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(54) Title: MUTATIONS IN ION CHANNELS

(57) Abstract: A method of identifying a subject predisposed to a disorder associated with ion channel dysfunction, comprising ascertaining whether at least one of the genes encoding ion channel subunits in said subject has undergone a mutation event such that a cDNA derived from said subject has the sequence set forth in one of SEQ ID NOS: 1-134.

affected individuals (Gardiner, 2000). As epileptic seizures may be the end-point of a number of molecular aberrations that ultimately disturb neuronal synchrony, the genetic basis for epilepsy is likely to be heterogeneous. There are over 200 Mendelian diseases which include epilepsy as part of the phenotype. In these diseases, seizures are symptomatic of underlying neurological involvement such as disturbances in brain structure or function. In contrast, there are also a number of "pure" epilepsy syndromes in which epilepsy is the sole manifestation in the affected individuals. These are termed idiopathic and account for over 60% of all epilepsy cases.

Idiopathic epilepsies have been further divided into partial and generalized sub-types. Partial (focal or local) epileptic fits arise from localized cortical discharges, so that only certain groups of muscles are involved and consciousness may be retained (Sutton, 1990). However, in generalized epilepsy, EEG discharge shows no focus such that all subcortical regions of the brain are involved. Although the observation that generalized epilepsies are frequently inherited is understandable, the mechanism by which genetic defects, presumably expressed constitutively in the brain, give rise to partial seizures is less clear.

The molecular genetic era has resulted in spectacular advances in classification, diagnosis and biological understanding of numerous inherited neurological disorders including muscular dystrophies, familial neuropathies and spinocerebellar degenerations. These disorders are all uncommon or rare and have simple Mendelian inheritance. In contrast, common neurological diseases like epilepsy, have complex inheritance where they are determined by multiple genes sometimes interacting with environmental influences. Molecular genetic advances in disorders with complex inheritance have been far more modest to date (Todd, 1999).

and Baier, 1989; Greenberg et al., 1988a; 1992; Janz et al., 1992).

Two broad groups of IGE are now known - the classical idiopathic generalized epilepsies (Commission on Classification and Terminology of the International League Against Epilepsy, 1989) and the newly recognized genetic syndrome of generalized epilepsy with febrile seizures plus (GEFS$^+$) (Scheffer and Berkovic, 1997; Singh et al., 1999).

The classical IGEs are divided into a number of clinically recognizable but overlapping sub-syndromes including childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy etc (Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Roger et al., 1992). The sub-syndromes are identified by age of onset and the pattern of seizure types (absence, myoclonus and tonic-clonic). Some patients, particularly those with tonic-clonic seizures alone do not fit a specifically recognized sub-syndrome. Arguments for regarding these as separate syndromes, yet recognizing that they are part of a neurobiological continuum, have been presented previously (Berkovic et al. 1987; 1994; Reutens and Berkovic, 1995).

GEFS$^+$ was originally recognized through large multi-generation families and comprises a variety of sub-syndromes. Febrile seizures plus (FS$^+$) is a sub-syndrome where children have febrile seizures occurring outside the age range of 3 months to 6 years, or have associated febrile tonic-clonic seizures. Many family members have a phenotype indistinguishable from the classical febrile convulsion syndrome and some have FS$^+$ with additional absence, myoclonic, atonic, or complex partial seizures. The severe end of the GEFS$^+$ spectrum includes myoclonic-astatic epilepsy.

The cumulative incidence for epilepsy by age 30 years (proportion suffering from epilepsy at some time) is 1.5% (Hauser et al., 1993). Accurate estimates for the

Italian League Against Epilepsy Genetic Collaborative Group, 1993).

Within single families with classical IGE or GEFS$^+$, affected individuals often have different sub-syndromes. The closer an affected relative is to the proband, the more similar are their sub-syndromes, and siblings often have similar sub-syndromes (Italian League Against Epilepsy Genetic Collaborative Group, 1993). Less commonly, families are observed where most, or all, known affected individuals have one classical IGE sub-syndrome such as childhood absence epilepsy or juvenile myoclonic epilepsy (Italian League Against Epilepsy Genetic Collaborative Group, 1993).

Importantly, sub-syndromes are identical in affected monozygous twins with IGE. In contrast, affected dizygous twins, may have the same or different sub-syndromes. Classical IGE and GEFS$^+$ sub-syndromes tend to segregate separately (Singh et al., 1999).

In some inbred communities, pedigree analysis strongly suggests recessive inheritance for juvenile myoclonic epilepsy and other forms of IGE (Panayiotopoulos and Obeid, 1989; Berkovic et al., 2000). In such families, sub-syndromes are much more similar in affected siblings than in affected sib-pairs from outbred families. Recently, a family with an infantile form of IGE with autosomal recessive inheritance, confirmed by linkage analysis, was described in Italy (Zara et al., 2000).

Most work on the molecular genetics of classical IGEs has been done on the sub-syndrome of juvenile myoclonic epilepsy where a locus in proximity or within the HLA region on chromosome 6p was first reported in 1988 (Greenberg et al., 1988*b*). This finding was supported by two collaborating laboratories, in separate patient samples, and subsequently three groups provided further evidence for a 6p locus for juvenile myoclonic epilepsy in some, but not all, of their families. However, genetic defects have not been found and the exact locus of the

be determined. Most of the studies reviewed above used analysis methods assuming Mendelian inheritance, an assumption that is not correct for outbred communities. Some studies used multiple models (autosomal recessive, autosomal dominant). Although parametric linkage analysis may be reliable in some circumstance of analyzing complex disease, it can lead to spurious findings as highlighted by the literature on linkage in major psychoses (Risch and Botstein, 1996).

In so far as GEFS$^+$ is concerned, linkage analysis on rare multi-generation large families with clinical evidence of a major autosomal dominant gene have demonstrated loci on chromosomes 19q and 2q. Both the 19q and 2q GEFS$^+$ loci have been confirmed in independently ascertained large families, and genetic defects have been identified. Families linked to 19q are known and a mutation in the gene for the β1 subunit of the neuronal sodium channel (SCN1B) has been identified (Wallace et al., 1998). This mutation results in the loss of a critical disulphide bridge of this regulatory subunit and causes a loss of function *in vitro*. Families linked to 2q are also known and mutations in the pore-forming α subunit of the neuronal sodium channel (SCN1A) have been identified (Australian provisional patent PR2203; Wallace et al., 2001*b*; Escayg et al., 2000). Studies on the more common small families with GEFS$^+$ have not revealed these or other mutations to date.

In addition to the *SCN1B* and *SCN1A* mutations in GEFS$^+$, four other gene defects have been discovered for human idiopathic epilepsies through the study of large families. Mutations in the alpha-4 subunit of the neuronal nicotinic acetylcholine receptor (CHRNA4) occur in the focal epilepsy syndrome of autosomal dominant nocturnal frontal lobe epilepsy (Australian patent AU-B-56247/96; Steinlein et al., 1995). Mutations in the gamma-2 subunit of the $GABA_A$ receptor (GABRG2) have been identified in childhood absence epilepsy, febrile seizures (including febrile

voltage-gated sodium, potassium, calcium and chloride channels as well as ligand-gated channels such as the acetylcholine and GABA receptors may lead to physiological disorders such as hyper- and hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia and cardiac arrhythmias. Neurological disorders other than epilepsy that are associated with ion channel mutations include episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, as well as neuropathic pain, inflammatory pain and chronic/acute pain. Some kidney disorders such as Bartter's syndrome, polycystic kidney disease and Dent's disease, secretion disorders such as hyperinsulinemic hypoglycemia of infancy and cystic fibrosis, and vision disorders such as congenital stationary night blindness and total colour-blindness may also be linked to mutations in ion channels.

Disclosure of the Invention

In a new genetic model for the idiopathic generalised epilepsies (IGEs) described in PCT/AU01/00872 (the disclosure of which is incorporated herein by reference) it has been postulated that most classical IGE and GEFS$^+$ cases are due to the combination of two mutations in multi-subunit ion channels. These are typically point mutations resulting in a subtle change of function. The critical postulate is that two mutations, usually, but not exclusively, in different subunit alleles ("digenic model"), are required for clinical expression of IGE. It was further proposed that

a) A number of different mutated subunit pairs can be responsible for IGE. Combinations of two mutated subunits lead to an IGE genotype with ~30% penetrance.

b) The total allele frequency of mutated subunits is ~8%. It was calculated that approximately 15% of the population has one or more mutated

an isolated nucleic acid molecule coding for a protein having a biological function as part of an ion channel in a mammal, wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred so as to affect the functioning of the ion channel. In some instances this single mutation alone will produce a phenotype of epilepsy or other neuro/physiological disorders associated with ion channel dysfunction.

In the case where a single mutation alone does not produce, say, an epilepsy phenotype, there would be provided one or more additional isolated nucleic acid molecules coding for proteins having a biological function as part of an ion channel in a mammal, wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred so as to affect the functioning of the ion channel. The cumulative effect of the mutations in each isolated nucleic acid molecule *in vivo* is to produce a epilepsy or another neuro/physiological disorders in said mammal. The mutations may be in nucleic acid molecules coding for protein subunits belonging to the same ion channel or may be in nucleic acid molecules coding for protein subunits that belong to different ion channels.

Typically such mutations are point mutations and the ion channels are voltage-gated channels such as a sodium, potassium, calcium or chloride channels or are ligand-gated channels such as members of the nAChR/GABA super family of receptors, or a functional fragment or homologue thereof.

Mutations may include those in non-coding regions of the ion channel subunits (eg mutations in the promoter region which affect the level of expression of the subunit gene, mutations in intronic sequences which affect the correct splicing of the subunit during mRNA processing, or mutations in the 5′ or 3′ untranslated regions that can affect translation or stability of the mRNA). Mutations

provided a combination of two or more isolated nucleic acid molecules each having a novel mutation event as laid out in Table 1. The cumulative effect of the mutations in each isolated nucleic acid molecule *in vivo* is to produce an epilepsy or another disorder associated with ion channel dysfunction as described above in said mammal.

In a particularly preferred embodiment of the present invention, the isolated nucleic acid molecules have a nucleotide sequence as shown in any one of SEQ ID Numbers: 1-134. The sequences correspond to the novel DNA mutations or variants laid out in Table 1.

In another aspect of the present invention there is provided an isolated nucleic acid molecule comprising any one of the nucleotide sequences set forth in SEQ ID NOS: 1-134.

In another aspect of the present invention there is provided an isolated nucleic acid molecule consisting of any one of the nucleotide sequences set forth in SEQ ID NOS: 1-134.

In another aspect of the present invention there is provided an isolated nucleic acid molecule encoding a mutant subunit of a mammalian nicotinic acetylcholine receptor (nAchR), wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred in the nucleotides outside of the M2 domain of the subunit of said mammalian nicotinic acetylcholine receptor, so as to produce an epilepsy phenotype.

Preferably said mutation event is a point mutation.

In one form of the invention, the mutations are in exon 5 of the CHRNA4 subunit and result in the replacement of an arginine residue with a cysteine residue at amino acid position 336, the replacement of an arginine residue with a glutamine residue at amino acid position 369, or the replacement of a proline residue with an arginine residue at amino acid position 474. The R336C mutation lies in the intracellular loop and occurs as a result of a

expression of the gene product. PCR reassembly of gene fragments and the use of synthetic oligonucleotides allow the engineering of the nucleotide sequences of the present invention. For example, oligonucleotide-mediated site-directed mutagenesis can introduce further mutations that create new restriction sites, alter expression patterns and produce splice variants etc.

As a result of the degeneracy of the genetic code, a number of polynucleotide sequences, some that may have minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention includes each and every possible variation of a polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequences of the present invention, and all such variations are to be considered as being specifically disclosed.

The nucleic acid molecules of this invention are typically DNA molecules, and include cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified, or may contain non-natural or derivatised nucleotide bases as will be appreciated by those skilled in the art. Such modifications include labels, methylation, intercalators, alkylators and modified linkages. In some instances it may be advantageous to produce nucleotide sequences possessing a substantially different codon usage than that of the polynucleotide sequences of the present invention. For example, codons may be selected to increase the rate of expression of the peptide in a particular prokaryotic or eukaryotic host corresponding with the frequency that particular codons are utilized by the host. Other reasons to alter the nucleotide sequence without altering the encoded amino acid sequences include the production of RNA transcripts

different ion channels the host cells will express two or more mutant receptor proteins. Typically said host cells are transfected with an expression vector comprising a DNA molecule according to the invention or, in particular, DNA molecules encoding two or more mutant ion channel subunits. A variety of expression vector/host systems may be utilized to contain and express sequences encoding polypeptides of the invention. These include, but are not limited to, microorganisms such as bacteria transformed with plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); or mouse or other animal or human tissue cell systems. Mammalian cells can also be used to express a protein using a vaccinia virus expression system. The invention is not limited by the host cell or vector employed.

The polynucleotide sequences, or variants thereof, of the present invention can be stably expressed in cell lines to allow long term production of recombinant proteins in mammalian systems. Sequences encoding the polypeptides of the present invention can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. The selectable marker confers resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode a protein may be designed to

Fragments of the polypeptides of the present invention may also be produced by direct peptide synthesis using solid-phase techniques. Automated synthesis may be achieved by using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Various fragments of this protein may be synthesized separately and then combined to produce the full-length molecule.

The present invention is also concerned with polypeptides having a biological function as an ion channel in a mammal, wherein a mutation event selected from the group consisting of substitutions, deletions, truncations, insertions and rearrangements has occurred so as to affect the functioning of the ion channel. In some instances this single mutation alone will produce an epilepsy phenotype.

In the case where a single mutation alone does not produce an epilepsy phenotype, there would be provided one or more additional isolated mammalian polypeptides having biological functions as part of an ion channel in a mammal, wherein a mutation event selected from the group consisting of substitutions, deletions, truncations, insertions and rearrangements has occurred so as to affect the functioning of the ion channel. The cumulative effect of the mutations in each isolated mammalian polypeptide *in vivo* being to produce an epilepsy in said mammal. The mutations may be in polypeptide subunits belonging to the same ion channel as described above, but may also be in polypeptide subunits that belong to different ion channels.

Typically the mutation is an amino acid substitution and the ion channel is a voltage-gated channel such as a sodium, potassium, calcium or chloride channel or a ligand-gated channel such as a member of the nAChR/GABA super family of receptors, or a functional fragment or homologue thereof.

Mutation combinations may be selected from, but are not restricted to, those represented in Table 1.

According to still another aspect of the present invention there is provided an isolated polypeptide comprising the amino acid sequence set forth in any one of SEQ ID NOS: 135-173.

According to still another aspect of the present invention there is provided a polypeptide consisting of the amino acid sequence set forth in any one of SEQ ID NOS: 135-173.

According to still another aspect of the present invention there is provided an isolated polypeptide, said polypeptide being a mutant subunit of a mammalian nicotinic acetylcholine receptor (nAChR), wherein a mutation event selected from the group consisting of substitutions, deletions, insertions and rearrangements has occurred outside of the M2 domain, so as to produce an epilepsy phenotype.

In one form of the invention the mutations are located in the intracellular loop of the CHRNA4 subunit and are substitutions in which an arginine residue is replaced with a cysteine residue, an arginine residue is replaced with a glutamine, or a proline residue is replaced with an arginine. Preferably the substitutions are R336C, R369Q and P474R transitions as illustrated by SEQ ID NOS: 153, 154 and 155 respectively.

In a further form of the invention, the mutation event is a substitution in which a threonine residue is replaced with a methionine residue in the signal sequence of CHRNB2. Preferably the substitution is a T26M transition as illustrated in SEQ ID NO: 156.

In a still further form of the invention, the mutation events are substitutions in which a leucine residue is replaced with a valine residue, or a valine residue is replaced with an alanine located in the M3 domain of CHRNB2. Preferably the substitutions are a L301V or V308A transition as illustrated in SEQ ID NOS: 157 and 173.

In a still further form of the invention, the

detect the presence of either a normal or mutated gene or gene product. The invention enables therapeutic methods for the treatment of epilepsy as well as other disorders associated with ion channel dysfunction and also enables methods for the diagnosis of epilepsy as well as other disorders associated with ion channel dysfunction.

Therapeutic Applications

According to still another aspect of the invention there is provided a method of treating epilepsy as well as other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness, comprising administering a selective antagonist, agonist or modulator of an ion channel or ion channel subunit, when the ion channel contains a mutation in a subunit comprising the channel, as described above, to a subject in need of such treatment. Said mutation event may be causative of the disorder when expressed alone or when expressed in combination with one or more additional mutations in subunits of the same or different ion channels, which are typically those identified in Table 1.

In still another aspect of the invention there is provided the use of a selective antagonist, agonist or modulator of an ion channel or ion channel subunit when the ion channel contains a mutation in a subunit comprising the channel, as described above, said mutation being causative of epilepsy as well as other disorders associated with ion channel dysfunction, including but not

another disorder associated with ion channel dysfunction when expressed alone or when expressed in combination with one or more other mutations in subunits of the same or different ion channels. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies as would be understood by the person skilled in the art.

For the production of antibodies, various hosts including rabbits, rats, goats, mice, humans, and others may be immunized by injection with a polypeptide as described above or with any fragment or oligopeptide thereof which has immunogenic properties. Various adjuvants may be used to increase immunological response and include, but are not limited to, Freund's, mineral gels such as aluminium hydroxide, and surface-active substances such as lysolecithin. Adjuvants used in humans include BCG (bacilli Calmette-Guerin) and Corynebacterium parvum.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to the mutant ion channel have an amino acid sequence consisting of at least 5 amino acids, and, more preferably, of at least 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of ion channel amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to a mutant ion channel may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (For example, see Kohler et al., 1975; Kozbor et al., 1985; Cote et al., 1983; Cole et al., 1984).

infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness, comprising administering an isolated DNA molecule which is the complement (antisense) of any one of the DNA molecules described above and which encodes an RNA molecule that hybridizes with the mRNA encoding a mutant ion channel subunit of the invention, to a subject in need of such treatment.

Typically, a vector expressing the complement (antisense) of the polynucleotides of the invention may be administered to a subject in need of such treatment. Antisense strategies may use a variety of approaches including the use of antisense oligonucleotides, injection of antisense RNA, ribozymes, DNAzymes and transfection of antisense RNA expression vectors. Many methods for introducing vectors into cells or tissues are available and equally suitable for use *in vivo*, *in vitro*, and *ex vivo*. For *ex vivo* therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (For example, see Goldman *et al.*, 1997).

In a still further aspect of the invention there is provided the use of an isolated DNA molecule which is the complement (antisense) of a DNA molecule of the invention and which encodes an RNA molecule that hybridizes with the mRNA encoding a mutant ion channel subunit of the invention, in the manufacture of a medicament for the treatment of epilepsy as well as other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive

approaches as described above for complement administration.

There is therefore provided a method of treating epilepsy as well as other disorders associated with ion channel dysfunction comprising administration of an antibody or complement to a mutant ion channel or ion channel subunit of the invention in combination with administration of wild-type ion channel subunits.

In still another aspect of the invention there is provided the use of an antibody or complement to a mutant ion channel or ion channel subunit of the invention in combination with the use of wild-type ion channel subunits, in the manufacture of a medicament for the treatment of epilepsy as well as other disorders associated with ion channel dysfunction.

In further embodiments, any of the agonists, antagonists, modulators, antibodies, complementary sequences or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents may be made by those skilled in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, therapeutic efficacy with lower dosages of each agent may be possible, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Drug Screening

According to still another aspect of the invention, peptides of the invention, particularly purified mutant ion channel subunit polypeptide and cells expressing these, are useful for the screening of candidate

transformed cells, transfected or injected oocytes, or animal models bearing mutated ion channel subunits such as transgenic animals or gene targeted (knock-in) animals (see transformed hosts). Drug candidates can be added to cultured cells that express a single mutant ion channel subunit or combination of mutant ion channel subunits (appropriate wild-type ion channel subunits should also be expressed for receptor assembly), can be added to oocytes transfected or injected with either a mutant ion channel subunit or combination of mutant ion channel subunits (appropriate wild-type ion channel subunits must also be injected for receptor assembly), or can be administered to an animal model containing a mutant ion channel or combination of mutant ion channels. Determining the ability of the test compound to modulate mutant ion channel activity can be accomplished by a number of techniques known in the art. These include for example measuring the effect on the current of the channel (e.g. calcium-, chloride-, sodium-, potassium-ion flux) as compared to the current of a cell or animal containing wild-type ion channels. Current in cells can be measured by a number of approaches including the patch-clamp technique (methods described in Hamill *et al*, 1981) or using fluorescence based assays as are known in the art (see Gonzalez et al. 1999). Drug candidates that alter the current to a more normal level are useful for treating or preventing epilepsy as well as other disorders associated with ion channel dysfunction.

Another technique for drug screening provides high-throughput screening for compounds having suitable binding affinity to the mutant ion channel subunit polypeptides of the invention or ion channels containing these (see PCT published application WO84/03564). In this stated technique, large numbers of small peptide test compounds can be synthesised on a solid substrate (such as a micotitre plate) and can be assayed for mutant ion channel subunit polypeptide or mutant ion channel binding. Bound

mimic the pharmacophore can be added. The selection can be made such that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, does not degrade *in vivo* and retains the biological activity of the lead compound. Further optimisation or modification can be carried out to select one or more final mimetics useful for *in vivo* or clinical testing.

It is also possible to isolate a target-specific antibody and then solve its crystal structure. In principle, this approach yields a pharmacophore upon which subsequent drug design can be based as described above. It may be possible to avoid protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analogue of the original receptor. The anti-id could then be used to isolate peptides from chemically or biologically produced peptide banks.

One superior method for drug screening relies on structure-based rational drug design. Determination of the three dimensional structure of the polypeptides of the invention, or the three dimensional structure of the ion channels which incorporate these polypeptides allows for structure-based drug design to identify biologically active lead compounds.

Three dimensional structural models can be generated by a number of applications, some of which include experimental models such as x-ray crystallography and NMR and/or from *in silico* studies of structural databases such as the Protein Databank (PDB). In addition, three dimensional structural models can be determined using a number of known protein structure prediction techniques based on the primary sequences of the polypeptides (e.g. SYBYL - Tripos Associated, St. Louis, MO), *de novo* protein structure design programs (e.g. MODELER - MSI Inc., San Diego, CA, or MOE - Chemical Computing Group, Montreal,

to that amount of the compound sufficient to result in amelioration of symptoms of the disorder.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The data obtained from these studies can then be used in the formulation of a range of dosages for use in humans.

Pharmaceutical compositions for use in accordance with the present invention can be formulated in a conventional manner using one or more physiological acceptable carriers, excipients or stabilisers which are well known. Acceptable carriers, excipients or stabilizers are non-toxic at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including absorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; binding agents including hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or non-ionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

The formulation of pharmaceutical compositions for use in accordance with the present invention will be based on the proposed route of administration. Routes of administration may include, but are not limited to, inhalation, insufflation (either through the mouth or nose), oral, buccal, rectal or parental administration.

Diagnostic Applications

Polynucleotide sequences encoding an ion channel subunit may be used for the diagnosis of epilepsy, as well as other as other disorders associated with ion channel

is provided the use of a polypeptide as described above in the diagnosis of epilepsy as well as other as other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

When a diagnostic assay is to be based upon proteins constituting an ion channel, a variety of approaches are possible. For example, diagnosis can be achieved by monitoring differences in the electrophoretic mobility of normal and mutant proteins that form the ion channel. Such an approach will be particularly useful in identifying mutants in which charge substitutions are present, or in which insertions, deletions or substitutions have resulted in a significant change in the electrophoretic migration of the resultant protein. Alternatively, diagnosis may be based upon differences in the proteolytic cleavage patterns of normal and mutant proteins, differences in molar ratios of the various amino acid residues, or by functional assays demonstrating altered function of the gene products.

In another aspect, antibodies that specifically bind mutant ion channels may be used for the diagnosis of a disorder, or in assays to monitor patients being treated with a complete ion channel or agonists, antagonists, modulators or inhibitors of an ion channel. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for ion channels include methods that utilize the antibody and a label to detect a mutant ion channel in

to develop and monitor the activities of therapeutic agents.

According to a further aspect of the present invention, tissue material obtained from animal models generated as a result of the identification of specific ion channel subunit human mutations (see below), particularly those disclosed in the present invention, can be used in microarray experiments. These experiments can be conducted to identify the level of expression of specific ion channel subunits, or any cDNA clones from whole-tissue libraries, in diseased tissue as opposed to normal control tissue. Variations in the expression level of genes, including ion channel subunits, between the two tissues indicates their possible involvement in the disease process either as a cause or consequence of the original ion channel subunit mutation present in the animal model. These experiments may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents. Microarrays may be prepared, used, and analyzed using methods known in the art. (For example, see Schena et al., 1996; Heller et al., 1997).

Transformed Hosts

The present invention also provides for the production of genetically modified (knock-out, knock-in and transgenic), non-human animal models transformed with nucleic acid molecules containing the novel ion channel mutations or variants as laid out in Table 1. These animals are useful for the study of the function of ion channels, to study the mechanisms by which combinations of mutations in ion channel subunits interact to give rise to disease and the effects of these mutations on tissue development, for the screening of candidate pharmaceutical compounds, for the creation of explanted mammalian cell cultures which express mutant ion channels or combinations

channel subunit gene, homologous recombination using embryonic stem cells may be applied.

For oocyte injection, one or more copies of the mutant ion channel subunit gene, or combinations thereof, can be inserted into the pronucleus of a just-fertilized mouse oocyte. This oocyte is then reimplanted into a pseudo-pregnant foster mother. The liveborn mice can then be screened for integrants using analysis of tail DNA or DNA from other tissues for the presence of the particular human subunit gene sequence. The transgene can be either a complete genomic sequence injected as a YAC, BAC, PAC or other chromosome DNA fragment, a complete cDNA with either the natural promoter or a heterologous promoter, or a minigene containing all of the coding region and other elements found to be necessary for optimum expression.

Once animals have been produced which contain a specific mutation in a particular ion channel subunit, mating combinations may be initiated between such animals so as to produce progeny containing combinations of two or more ion channel mutations. These animals effectively mimic combinations of mutations that are proposed here to cause human IGE cases. These animal models can subsequently be used to study the extent and mechanisms of disease as related to the mutated ion channel combinations, as well as for the screening of candidate therapeutic compounds.

According to still another aspect of the invention there is provided the use of genetically modified non-human animals as described above for the screening of candidate pharmaceutical compounds (see drug screening above). These animals are also useful for the evaluation (eg therapeutic efficacy, toxicity, metabolism) of candidate pharmaceutical compounds, including those identified from the invention as described above, for the treatment of epilepsy as well as other as other disorders associated with ion channel dysfunction as described above.

identified in the ion channel subunits constituting the sodium channel. These examples include both novel and previously identified mutations;

Figure 3 provides examples of epilepsy pedigrees where mutation profiles of ion channel subunits for individuals constituting the pedigree have begun to be determined. These examples have been used to illustrate how the identification of novel ion channel subunit mutations and variations in IGE individuals can combine to give rise to the disorder.

Modes for Performing the Invention

Potassium channels are the most diverse class of ion channel. The *C. elegans* genome encodes about 80 different potassium channel genes and there are probably more in mammals. About ten potassium channel genes are known to be mutated in human disease and include four members of the KCNQ gene sub-family of potassium channels. KCNQ proteins have six transmembrane domains, a single P-loop that forms the selectivity filter of the pore, a positively charged fourth transmembrane domain that probably acts as a voltage sensor and intracellular amino and carboxy termini. The C terminus is long and contains a conserved "A domain" followed by a short stretch thought to be involved in subunit assembly.

Four KCNQ subunits are thought to combine to form a functional potassium channel. All five known KCNQ proteins can form homomeric channels *in vitro* and the formation of heteromers appears to be restricted to certain combinations.

Sodium (the alpha subunit) and calcium channels are thought to have evolved from the potassium channel subunit, and they each consist of four domains covalently linked as the one molecule, each domain being equivalent to one of the subunits that associate to form the potassium channel. Each of the four domains of the sodium and calcium channels are comprised of six transmembrane

subunits.

Neuronal nicotinic acetylcholine receptors (nAChRs) consist of heterologous pentamers comprising various combinations of alpha subunits or alpha and beta subunits ($\alpha2$-$\alpha9$; $\beta2$-$\beta4$). The alpha subunits are characterised by adjacent cysteine residues at amino acid positions 192 and 193, and the beta subunits by the lack of these cysteine residues. They are ligand-gated ion channels differentially expressed throughout the brain to form physiologically and pharmacologically distinct receptors hypothesised to mediate fast, excitatory transmission between neurons of the central nervous system or to modulate neurotransmission from their presynaptic position.

In chicken and rat, the predominant nAChR subtype is composed of alpha-4 and beta-2 subunits. The transmembrane 2 (M2) segments of the subunits are arranged as alpha helices and contribute to the walls of the neurotransmitter-gated ion channel. The alpha helices appear to be kinked and orientated in such a way that the side chains of the highly conserved M2-leucine residues project inwards when the channel is closed. ACh is thought to cause a conformational change by altering the association of the amino acid residues of M2. The opening of the channel seems to be due to rotations of the gate forming side chains of the amino acid residues; the conserved polar serines and threonines may form the critical gate in the open channel.

Example 1: Identification of mutations in ion channels

Previous studies by reference (Wallace et al., 1998; PCT/AU01/00581; Wallace et al., 2001*b*; Australian patent AU-B-56247/96; Steinlein et al., 1995; PCT/AU01/00541; Phillips et al., 2001; PCT/AU01/00729; PCT/AU01/01648; Wallace et al., 2001a, the disclosures of which are incorporated herein by reference) have identified mutations in a number of ion channel subunits associated

Example 2: Sample preparation for SSCP screening

A large collection of individuals affected with epilepsy have undergone careful clinical phenotyping and additional data regarding their family history has been collated. Informed consent was obtained from each individual for blood collection and its use in subsequent experimental procedures. Clinical phenotypes incorporated classical IGE cases as well as GEFS+ and febrile seizure cases.

DNA was extracted from collected blood using the QIAamp DNA Blood Maxi kit (Qiagen) according to manufacturers specifications or through procedures adapted from Wyman and White (1980). Stock DNA samples were kept at a concentration of 1 ug/ul.

In preparation for SSCP analysis, samples to be screened were formatted into 96-well plates at a concentration of 30 ng/ul. These master plates were subsequently used to prepare exon specific PCR reactions in the 96-well format.

Example 3: Identification of sequence alterations in ion channel genes

SSCP analysis of specific ion channel exons followed by sequencing of SSCP bandshifts was performed on individuals constituting the 96-well plates to identify sequence alterations.

Primers used for SSCP were labelled at their 5′ end with HEX and typical PCR reactions were performed in a total volume of 10 µl. All PCR reactions contained 67 mM Tris-HCl (pH 8.8); 16.5 mM $(NH_4)_2SO_4$; 6.5 µM EDTA; 1.5 mM $MgCl_2$; 200 µM each dNTP; 10% DMSO; 0.17 mg/ml BSA; 10 mM β-mercaptoethanol; 5 µg/ml each primer and 100 U/ml *Taq* DNA polymerase. PCR reactions were performed using 10 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds followed by 25 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. A final

possible effects on the receptor physiological properties. To examine possible receptor modifications, expression experiments in *Xenopus* oocytes were designed. However, because it is known that all patients that carry this mutation are heterozygous all experiments were carried out by co-expression of equal amounts of the patient control and mutated cDNA. Amplitude of the ACh-evoked currents evoked by saturating agonist concentrations showed no significant difference between the control and heterozygous expression. A marked difference was, however, observed when examining the receptor sensitivity to acetylcholine (ACh). Mutant containing receptors display a greater response at low ACh concentrations than their control counterpart. As previous functional studies of the first CHRNA4 mutation (S248F, Phillips et al., 1995) showed an increased receptor desensitization, the time course of the response for the I225S mutation was thoroughly monitored. Results showed that the average time course of the ACh-evoked current was not significantly different from the control and thereby suggests a minimal effect of this M1 mutation on the receptor desensitization properties.

Mutation screening by SSCP analysis of ADNFLE affected individuals has lead to the identification of a further 3 mutations in the CHRNA4 subunit gene that fall outside of the M2 domain (R336C, R369Q and P474R) as well as 4 new CHRNB2 mutations that also lie outside the M2 domain (T26M, L301V, V308A and G412D). To test the functional significance of these mutations the L301V CHRNB2 mutation was examined in *Xenopus* oocytes using similar approaches as for the I225S mutation in CHRNA4.

Results from these experiments showed that the L301V mutation caused no significant changes at the current amplitude evoked by saturating ACh concentration but a pronounced increased in agonist sensitivity was observed at the dose-response curve. In addition, the time course

changes in functional properties with either the disease penetrance or severity are, however, difficult to make in view of the restricted number of cases affected by each mutation.

Modification of the ACh sensitivity can be accompanied by an alteration of the response time course. Two possibilities can be envisaged with either an increase or a decrease in the desensitization profile. Examples of increased desensitization have already been reported for the CHRNA4 S248F or 776ins3 mutants (Bertrand et al., 1998). Averaging normalized responses of the CHRNA4 I225S mutant receptor readily illustrates that no significant modification of the response time course can be observed versus the controls recorded in the same batch of oocytes. In contrast, a significant reduction of the desensitization time course is observed for the CHRNB2 L301V mutant. Moreover, computing the maximal ACh evoked currents for the control and the L301V mutant revealed that cells expressing this mutant receptor display larger currents. While at present this increase in mean current can be attributed either to a higher level of protein expression or to a difference in single channel properties these data suggest that the L301V mutation causes a gain of function.

The number of distinct mutations associated with ADNFLE further illustrates the importance of the nAChRs in the triggering of seizures of patients suffering from this form of epilepsy. Constituting the first report of mutations localized outside the critical M2 segment the nAChR subunit mutants functionally characterized herein present the typical common trait of an increase in ACh sensitivity. These results indicate that mutations may occur in many different segments of the protein and therefore largely extend the probability of spontaneous occurrence. This probability is even higher, given that a mutation in either the CHRNA4 or CHRNB2 subunit is

PCR products showing a conformational change were subsequently sequenced. This first involved re-amplification of the relevant amplicon using primers without the 5' HEX addition followed by purification of the PCR amplified templates for sequencing using QiaQuick PCR preps (Qiagen) based on manufacturers procedures. The primers used to sequence the purified nAChR subunit amplicons were identical to those used for the initial amplification step. For each sequencing reaction, 25 ng of primer and 100 ng of purified PCR template were used. The BigDye sequencing kit (ABI) was used for all sequencing reactions according to the manufacturers specifications. The products were run on an ABI 377 Sequencer and analysed using the EditView program.

The sequencing strategy revealed a number of nucleotide substitutions in both the CHRNA4 and CHRNB2 genes which were specific for affected individuals and not present in the normal population.

For CHRNA4, 3 epilepsy specific mutations were identified in the intracellular loop. These included a C→T transition at nucleotide position 1006 of the coding sequence, a G→A transition at nucleotide position 1106 of the coding sequence and a C→G transition at nucleotide position 1421 of the coding sequence. These nucleotide substitutions lead to R336C, R369Q and P474R amino acid changes respectively.

For CHRNB2, 4 epilepsy specific mutations were identified. These included a C→T transition at nucleotide position 77 of the coding sequence which lies in the signal sequence, a C→G transition at nucleotide position 901 and a T→C transition at nucleotide position 923 of the coding sequence which lie in the M3 domain, and a G→A transition at nucleotide position 1235 of the coding sequence. These nucleotide substitutions lead to T26M, L301V, V308A and G412D amino acid changes respectively.

Xenopus Oocyte expression

Using the mutations and variations in ion channel subunits that form part of this invention, the digenic model may be validated through a parametric analysis of large families in which two abnormal alleles co-segregate by chance to identify mutations which act co-operatively to give an epilepsy phenotype. It is envisaged that the strategy of careful clinical phenotyping in these large families, together with a linkage analysis based on the digenic hypothesis will allow identification of the mutations in ion channels associated with IGEs. If molecular genetic studies in IGE are successful using the digenic hypothesis, such an approach might serve as a model for other disorders with complex inheritance.

The digenic hypothesis predicts that the closer the genetic relationship between affected individuals, the more similar the sub-syndromes, consistent with published data (Italian League Against Epilepsy Genetic Collaborative Group, 1993). This is because more distant relatives are less likely to share the same combinations of mutated subunits.

Identical twins have the same pair of mutated subunits and the same minor alleles so the sub-syndromes are identical. Affected sib-pairs, including dizygous twins, with the same sub-syndrome would also have the same pair of mutated subunits, but differences in minor alleles would lead to less similarity than with monozygous twins. Some sib-pairs and dizygous twins, have quite different sub-syndromes; this would be due to different combinations of mutated subunits, when the parents have more than two mutated alleles between them.

A special situation exists in inbred communities that parallels observations on autosomal recessive mouse models. Here the two mutated alleles of the digenic model are the same and thus result in a true autosomal recessive disorder. Because all affected individuals have the same pair of mutated alleles, and a similar genetic background, the phenotypes are very similar.

derive from a 2 x 0 and 0 x 2 matings etc. For the 2 x 0 and 0 x 2 matings, half the parents have IGE genotypes and contribute .16 (.33/2) to the parental risk with the total parental risk of an IGE genotype being .258. The other matings that contribute to affected parent-child pairs are 2 x 1, 1 x 2, 3 x 0, 0 x 3 etc.

The sibling risk of an IGE genotype is .305. For example 2 x 0 and 0 x 2 matings contributed .08 to the sibling risk (.33[fraction of children with 2 abnormal alleles] x .25[the chance of that mating producing a child with 2 or more abnormal alleles]). Similarly the offspring risk was determined to be .248 by mating individuals with 2 abnormal alleles with the general population. Thus at 30% penetrance the risk for IGE phenotype for parents of a proband is .077, for siblings .091, and for offspring .074.

It can be shown that affected sib pairs share the same abnormal allele pair in 85% of cases. This is because of all affected sib pairs 44% derive from 1 x 1 matings and 23% from 0 x 2 and 2 x 0 matings where all affected siblings have the same genotype. In contrast, 24% derive from 1 x 2 matings and 9% from 3 x 1 and 2 x 2 matings etc where affected sibling genotypes sometimes differ.

For affected parent-child pairs, genotypes are identical in only 58%. Of affected parent child pairs, 43% derive from 0 x 2 matings where gentoypes are identical, whereas 38% derive from 0 x 3 and 17% from 1 x 2 where the majority of crosses yield different affected genotypes.

Based on the digenic model it has been postulated that most classical IGE and GEFS$^+$ cases are due to the combination of two mutations in multi-subunit ion channels. These are typically point mutations resulting in a subtle change of function. The critical postulate is that two mutations, usually, but not exclusively, in different subunit alleles ("digenic model"), are required for clinical expression of IGE.

parent-child pairs. This would be most objectively measured by age of onset and seizure types.

Estimates for the risk of febrile seizures or IGE in relatives vary. The estimates range from 5%-10% for siblings, 4%-6% for offspring, 3%-6% for parents, and 2-3% for grandparents. Underestimation may occur because IGE manifest in youth, and parents and particularly grandparents may be unaware of seizures in themselves in younger years. This is particularly true where there was stigma associated with epilepsy and where the epilepsy may have been mild and unrecognized. Underestimation of sibling and offspring risks occurs when unaffected young children are counted, some of whom will develop IGE in adolescence. Overestimation may occur with misdiagnosis of seizures or inclusion of seizures unrelated to IGE (e.g. due to trauma or tumors)

In autosomal dominant models the risk to affected relatives reduces proportionally (50% for first degree relatives, 25% for second degree etc). For all oligogenic or polygenic models the risk decreases more quickly. For a digenic model with three loci, the risks are 9.1% for siblings, 7.4% for offspring, 7.7% for parents. Rigorous measurement of the familial recurrence rates, with careful phenotyping and age-corrected risk estimates could be compared with the predictions from the digenic model, and it is proposed to do this.

There is a small amount of information on IGE families regarding haplotype distribution. For example, there is some evidence for a locus on 8q as determined by parametric linkage in a single family (Fong et al., 1998) and by non-parametric analysis in multiple small families (Zara et al., 1995). Interestingly, in the latter study the 8q haplotype not infrequently came from the unaffected parent. This would be quite compatible with the digenic model and evaluation of other data sets in this manner could be used to test the hypothesis, and it is proposed to do this.

mutation analysis of ion channel genes in these individuals has been carried out as described above. In Table 1 there is provided an indication of novel genetic alterations so far identified through mutation analysis screening of these individuals. Figure 2 provides an example to indicate where some of these mutations have occurred with respect to the sodium channel genes.

The identification of novel mutations and variations in ion channel subunits in IGE individuals provides resources to further test the digenic hypothesis and mutation profiles are starting to accumulate for a number of subunit changes that are observed in the same individuals. Figure 3 provides results from some of these profiles.

Figure 3A shows a 3 generation family in which individual III-1 has myoclonic astatic epilepsy and contains a N43del mutation in the SCN3A gene as well as an A1067T mutation in the SCN1A gene. Individual I-1 also has the SCN3A mutation but alone this mutation is not sufficient to cause epilepsy in this individual. The SCN3A mutation has likely been inherited from the grandfather through the mother, while the SCN1A mutation is likely to arise from the father. Both parents are unaffected but have yet to be screened for the presence of the mutations in these subunits. Individual II-1 is likely to contain an as yet unidentified ion channel subunit mutation acting in co-operation with the SCN3A mutation already identified in this individual.

Figure 3B is another 3 generation family in which individual III-1 has myoclonic astatic epilepsy due to a combination of the same SCN3A and SCN1A mutations as above. However, in this family both parents have febrile seizures most likely due to the presence of just one of the mutations in each parent, as proposed by the model. This is in contrast to individuals II-2 and II-3 in Figure 4A who also contain one of the mutations in these genes each. These individuals are phenotypically normal most

expressed as a fusion to a peptide that has a DNA binding domain. A second gene, or number of genes, such as those from a cDNA library (TARGET), is cloned so that it is expressed as a fusion to an activation domain. Interaction of the protein of interest with its binding partner brings the DNA-binding peptide together with the activation domain and initiates transcription of the reporter genes. The first reporter gene will select for yeast cells that contain interacting proteins (this reporter is usually a nutritional gene required for growth on selective media). The second reporter is used for confirmation and while being expressed in response to interacting proteins it is usually not required for growth.

Ion channel interacting genes may also be targets for mutation in epilepsy as well as other disorders associated with ion channel dysfunction. A mutation in an ion channel interacting gene when expressed alone, or when expressed in combination with one or more other ion channel mutations or ion channel interacting gene mutations (based on the digenic model), may give rise to the disorder. The nature of the ion channel interacting genes and proteins can be studied such that these partners can also be targets for drug discovery.

Structural studies

Ion channel recombinant proteins can be produced in bacterial, yeast, insect and/or mammalian cells and used in crystallographical and NMR studies. Together with molecular modelling of the protein, structure-driven drug design can be facilitated.

Industrial Applicability

The mutant ion channel receptor subunits of the invention are useful in the diagnosis and treatment of diseases such as epilepsy and disorders associated with ion channel dysfunction including, but not limited to, hyper- or hypo-kalemic periodic paralysis, myotonias,

TABLE 1

Examples of mutations and variations identified in ion channel subunit genes

Subunit Gene	Exon/Intron	DNA Mutation	Amino Acid Change	SEQ ID NOS
Sodium Channel Subunits				
Coding exonic variants - amino acid change				
SCN1A^{r}	Exon 1	c111delC	P37fsX91	1, 135
SCN1A^{ra}	Exon 4	c563A→T	D188V	
SCN1A^{r}	Exon 9	c1342-c1352del	I448X	2, 136
SCN1A^{r}	Exon 20	c3976G→C	A1326P	3, 137
SCN1A^{ra}	Exon 21	c4057G→C	V1353L	
SCN1A^{r}	Exon 24	c4556C→T	P1519L	4, 138
SCN1A^{r}	Exon 26	c4905C→G	F1635L	5, 139
SCN1A^{ra}	Exon 26	c4968C→G	I1656M	
SCN1A^{r}	Exon 26	c5363-c5364ins	N1788fsX1796	6, 140
SCN1A^{r}	Exon 26	c5536-c5539delAAAC	S1846fsX1856	7, 141
SCN1A^{r}	Exon 26	c5643G→C	E1881D	8, 142
SCN1A^{r}	Exon 26	c5870A→G	E1957G	9, 143
SCN8A^{r}	Exon 14	c3148G→A	G1050S	10, 144
SCN1B^{ra}	Exon 3	c253C→T	R85C	
SCN1B^{ra}	Exon 3	c363C→G	C121W	
SCN1B^{r}	Exon 3	c367G→A	V123I	11, 145
SCN1B^{r}	Exon 3	c373C→T	R125C	12, 146
SCN2A^{r}	Exon 21	c3988C→T	L1330F	13, 147
SCN2A^{r}	Exon 25	c4687C→G	L1563V	14,

CHRNB2^r	Exon 5	c923T→C	V308A	134, 173
CHRNB2^r	Exon 5	c1235G→A	G412D	37, 158
CHRNB2^c	Exon 5	c1191G→C	Q397H	38, 159
Coding variants - no amino acid change				
CHRNA4^r	Exon 5	c978C→T	-	39
CHRNA4^r	Exon 5	c1104C→T	-	40
CHRNA4^r	Exon 5	c1635G→A	-	41
CHRNA4^c	Exon 1	c51G→A	-	42
CHRNA4^c	Exon 5	c1629C→T	-	43
CHRNA4^c	Exon 5	c1659G→A	-	44
CHRNB2^r	Exon 2	c78G→A	-	45
CHRNB2^r	Exon 2	c109C→T	-	46
CHRNB2^r	Exon 5	c825G→A	-	47
CHRNB2^c	Exon 5	c1233G→A	-	48
CHRNB2^r	Exon 6	c1482A→G	-	49
Non-coding variants				
CHRNA4^c	Intron 5	IVS5+11C→T	-	50
CHRNA4^c	Intron 5	IVS5+14G→A	-	51
CHRNB2^c	Intron 5	IVS5+14G→A	-	52

Potassium Channel Subunits

Coding exonic variants – amino acid change

KCNQ3^r	Exon 15	c2306C→A	P769H	53, 160
KCNQ2^c	Exon 15	c2255C→A	T752N	54, 161
Coding exonic variants - no amino acid change				
KCNQ5^r	Exon 14	c1869A→T	-	55
KCNQ2^c	Exon 6	c912C→T	-	56
KCNQ2^c	Exon 11	c1419C→G	-	57
KCNQ2^c	Exon 15	c2154T→A	-	58
KCNQ2^c	Exon 15	c2460G→A	-	59
KCNQ3^c	Exon 4	c660T→C	-	60
KCNQ3^c	Exon 4	c732T→C	-	61
KCNQ3^c	Exon 7	c1071C→G	-	62
Non-coding variants				

GABRB1[c]	Exon 8	c846A→G	-	88
GABRA1[c]	Exon 4	c156T→C	-	89
GABRD[c]	Exon 4	c330C→T	-	90
GABRD[c]	Exon 7	c816C→T	-	91
GABRD[c]	Exon 9	c1104C→T	-	92
GABRG2[c]	Exon 3	c315C→T	-	93
GABRG2[c]	Exon 5	c588T→C	-	94
GABRA2[c]	Exon 6	c396G→A	-	95
GABRA6[c]	Exon 8	c1005G→C	-	96
GABRA5[c]	Exon 8	c606T→C	-	97
GABRA5[c]	Exon 10	c975T→C	-	98
GABRG1[c]	Exon 3	c264A→G	-	99
GABRG1[c]	Exon 11	c1459G→A	-	100
GABRE[c]	Exon 2	c186G→A	-	101

GABA Receptor Subunits

Non-coding variants				
GABRA1[r]	Exon 2	c-53C→A	-	102
GABRA2[r]	5' UTR	c-(-9-10)delAG	-	103
GABRB2[r]	5' UTR	c-213G→A	-	104
GABRB2[r]	Intron 1	IVS1-(-8-9)insT	-	105
GABRA2[r]	Intron 9	IVS9+149G→T	-	106
GABRD[r]	Intron 4	IVS4+45delG	-	107
GABRD[r]	Intron 6	IVS6+92G→T	-	108
GABRD[r]	Intron 6	IVS6+73C→T	-	109
GABRG3[r]	Intron 5	IVS5+20C→T	-	110
GABRG2[r]	Intron 1	IVS1+12C→T	-	111
GABRB1[r]	Intron 2	IVS2-51C→A	-	112
GABRA5[r]	Intron 6	IVS6+10G→C	-	113
GABRA3[r]	Intron 5	IVS5+26-29delGTCT	-	114
GABRPi[r]	Intron 1	IVS1-85C→T	-	115
GABRPi[r]	Intron 4	IVS4-85T→A	-	116
GABRPi[r]	Intron 7	IVS7+8A→C	-	117
GABRA3[c]	Intron 3	IVS3-(19-20)insT	-	118
GABRA4[c]	Intron 1	IVS1-10delT	-	119
GABRA4[c]	Intron 1	IVS1-(10-11)insT	-	120
GABRA4[c]	Intron 1	IVS1-(10-11)delTT	-	121

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Claims

1. A method of identifying a subject predisposed to a disorder associated with ion channel dysfunction, comprising ascertaining whether at least one of the genes encoding ion channel subunits in said subject has undergone a mutation event such that a cDNA derived from said subject has the sequence set forth in one of SEQ ID NOS: 1-134.

2. A method as claimed in claim 1, wherein said mutation event disrupts the functioning of an assembled ion channel so as to produce an epilepsy phenotype in said subject.

3. A method as claimed in claim 1, wherein said mutation event disrupts the functioning of an assembled ion channel so as to produce one or more disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness and total colour-blindness in said subject.

4. A method as claimed in claim 1, wherein said mutation event disrupts the functioning of an assembled ion channel so as to produce an epilepsy phenotype when expressed in combination with one or more additional mutations or variations in said ion channel subunit genes.

5. A method as claimed in claim 1, wherein said mutation event disrupts the functioning of an assembled ion channel

chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness and total colour-blindness.

9. An isolated nucleic acid molecule encoding a mutant or variant ion channel subunit as claimed in claim 6, wherein said mutation event disrupts the functioning of an assembled ion channel so as to produce an epilepsy phenotype when expressed in combination with one or more additional mutations or variations in said ion channel subunit genes.

10. An isolated nucleic acid molecule encoding a mutant or variant ion channel subunit as claimed in claim 6, wherein said mutation event disrupts the functioning of an assembled ion channel so as to produce one or more disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness and total colour-blindness, when expressed in combination with one or more additional mutations or variations in said ion channel subunit genes.

11. An isolated nucleic acid molecule comprising any one of the nucleotide sequences set forth in SEQ ID NOS: 1-134.

subunit genes.

17. An isolated polypeptide, said polypeptide being a mutant or variant ion channel subunit as claimed in claim 13, wherein said mutation event disrupts the functioning of an assembled ion channel so as to produce one or more disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness and total colour-blindness, when expressed in combination with one or more additional mutations or variations in said ion channel subunit genes.

18. An isolated polypeptide comprising any one of the amino acid sequences set forth in SEQ ID NOS: 135-173.

19. An isolated polypeptide consisting of any one of the amino acid sequences set forth in SEQ ID NOS: 135-173.

20. An isolated polypeptide complex, said polypeptide complex being an assembled mammalian ion channel including an ion channel subunit comprising a polypeptide as defined in any one of claims 13 to 19.

21. An expression vector comprising a nucleic acid molecule as defined in any one of claims 6 to 12.

22. A cell transformed with at least one expression vector as defined in claim 21.

31. A method of treating epilepsy comprising administering an antibody as claimed in either one of claims 29 or 30 to a subject in need of such treatment.

32. The use of an antibody, as claimed in either one of claims 29 or 30, in the manufacture of a medicament for the treatment of epilepsy.

33. A method of treating a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness, comprising administering an antibody as claimed in either one of claims 29 or 30 to a subject in need of such treatment.

34. The use of an antibody, as claimed in either one of claims 29 or 30, in the manufacture of a medicament for the treatment of a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

39. A method of treating epilepsy comprising administering an isolated DNA molecule which is the complement (antisense) of a nucleic acid molecule as claimed in any one of claims 6 to 12 and which encodes an RNA molecule that hybridizes with the mRNA encoded by a nucleic acid molecule as claimed in any one of claims 6 to 12, to a subject in need of such treatment.

40. The use of a DNA molecule which is the complement of a nucleic acid molecule as claimed in any one of claims 6 to 12 and which encodes an RNA molecule that hybridizes with the mRNA encoded by a nucleic acid molecule as claimed in any one of claims 6 to 12, in the manufacture of a medicament for the treatment of epilepsy.

41. A method of treating a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total

administration of the wild-type ion channel subunit, to a subject in need of such treatment.

44. The use of an antibody, as claimed in claims 29 or 30, use of an agonist, antagonist or modulator of an ion channel when it has undergone a mutation event or combination of events as defined in any one of claims 1 to 10 or claims 13 to 17, or use of a DNA molecule which is the complement of a nucleic acid molecule as claimed in any one of claims 6 to 12 and which encodes an RNA molecule that hybridizes with the mRNA encoded by a nucleic acid molecule as claimed in any one of claims 6 to 12, in combination with the use of the wild-type ion channel subunit, in the manufacture of a medicament for the treatment of epilepsy.

45. A method of treating a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness, comprising administering an antibody, as claimed in either one of claims 29 or 30, administration of an agonist, antagonist or modulator of an ion channel when it has undergone a mutation event or combination of events as defined in any one of claims 1 to 10 or claims 13 to 17, or administration of a DNA molecule which is the complement of a nucleic acid molecule as claimed in any one of claims 6 to 12 and which encodes an RNA molecule that hybridizes with the mRNA encoded by a nucleic acid molecule as claimed in any one of claims 6 to

49. Use of a nucleic acid molecule as claimed in any one of claims 6 to 12 for the screening of candidate pharmaceutical agents useful for the treatment of a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

50. Use of a polypeptide as claimed in any one of claims 13 to 19 or claim 28, or a polypeptide complex as claimed in claim 20 for the screening of candidate pharmaceutical agents.

51. Use of a polypeptide as claimed in any one of claims 13 to 19 or claim 28, or a polypeptide complex as claimed in claim 20 for the screening of candidate pharmaceutical agents useful for the treatment of epilepsy.

52. Use of a polypeptide as claimed in any one of claims 13 to 19 or claim 28, or a polypeptide complex as claimed in claim 20 for the screening of candidate pharmaceutical agents useful for the treatment of a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's

59. A genetically modified, non-human animal which has been transformed with two or more isolated nucleic acid molecules as claimed in any one of claims 6 to 12.

60. A genetically modified non-human animal as claimed in either one of claims 58 or 59 in which the animal is selected from the group consisting of rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs and non-human primates such as monkeys and chimpanzees.

61. A method of producing a non-human transgenic animal containing a combination of two or more ion channel mutations, comprising the steps of:

(1) creating a non-human transgenic animal comprising a first nucleic acid molecule as claimed in any one of claims 6 to 12;

(2) creating one or more additional non-human, transgenic animals comprising a second nucleic acid molecule as claimed in any one of claims 6 to 12; and

(3) conducting mating combinations so as to produce progeny containing combinations of two or more ion channel mutations which effectively mimic combinations of ion channel mutations responsible for human disease.

62. A non-human, transgenic animal produced by the process of claim 61.

63. The use of a genetically modified non-human animal as claimed in any one of claims 58 to 60 or a non-human transgenic animal as claimed in claim 62 in the screening of candidate pharmaceutical compounds.

64. The use of a genetically modified non-human animal as claimed in any one of claims 58 to 60 or a non-human

infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

68. The use of a polypeptide as defined in any one of claims 13 to 17 or claim 28, or polypeptide complex as claimed in claim 20 in the diagnosis of epilepsy.

69. The use of a polypeptide as defined in any one of claims 13 to 17 or claim 28, or polypeptide complex as claimed in claim 20 in the diagnosis of a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

70. The use of an antibody as claimed in either one of claims 29 or 30 in the diagnosis of epilepsy.

71. The use of an antibody as claimed in either one of claims 29 or 30 in the diagnosis of a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic

74. A method as claimed in either one of claims 71 or 72 wherein the DNA fragments are subjected to restriction enzyme analysis.

75. A method as claimed in either one of claims 71 or 72 wherein the DNA fragments are subjected to SSCP analysis.

76. An isolated nucleic acid molecule encoding a mutant subunit of a mammalian nicotinic acetylcholine receptor (nAChR), wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred in the nucleotides outside of the M2 domain of the subunit of said mammalian nicotinic acetylcholine receptor, so as to produce an epilepsy phenotype.

77. An isolated nucleic acid molecule encoding a mutant subunit of a mammalian nicotinic acetylcholine receptor (nAChR), wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred in the nucleotides outside of the M2 domain of the subunit of said mammalian nicotinic acetylcholine receptor, so as to produce a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

receptor (nAChR), wherein a mutation event selected from the group consisting of substitutions, deletions, insertions and rearrangements has occurred outside of the M2 domain, so as to produce a disorder associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

86. An isolated polypeptide as claimed in either one of claims 84 or 85 wherein said mutant subunit is the CHRNA4 subunit.

87. An isolated polypeptide as claimed in claim 86 wherein said mutation event takes place in the intracellular loop of the CHRNA4 subunit.

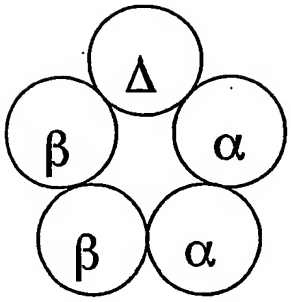
88. An isolated polypeptide as claimed in claim 86 wherein said mutation event is selected from the group consisting of an R336C mutation, R369Q mutation and P474R mutation.

89. An isolated polypeptide as claimed in either one of claims 84 or 85 wherein said mutant subunit is the CHRNB2 subunit.

90. An isolated polypeptide as claimed in claim 89 wherein said mutation event takes place in the signal sequence.

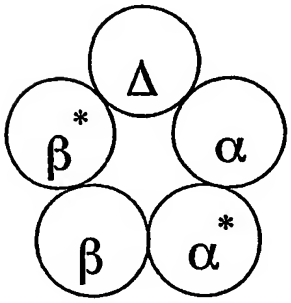
Figure 1

A



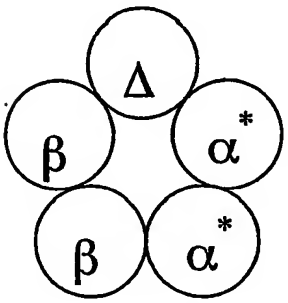
Normal

B



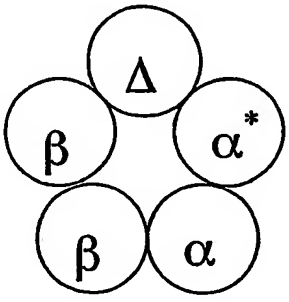
IGE: Outbred Population

C



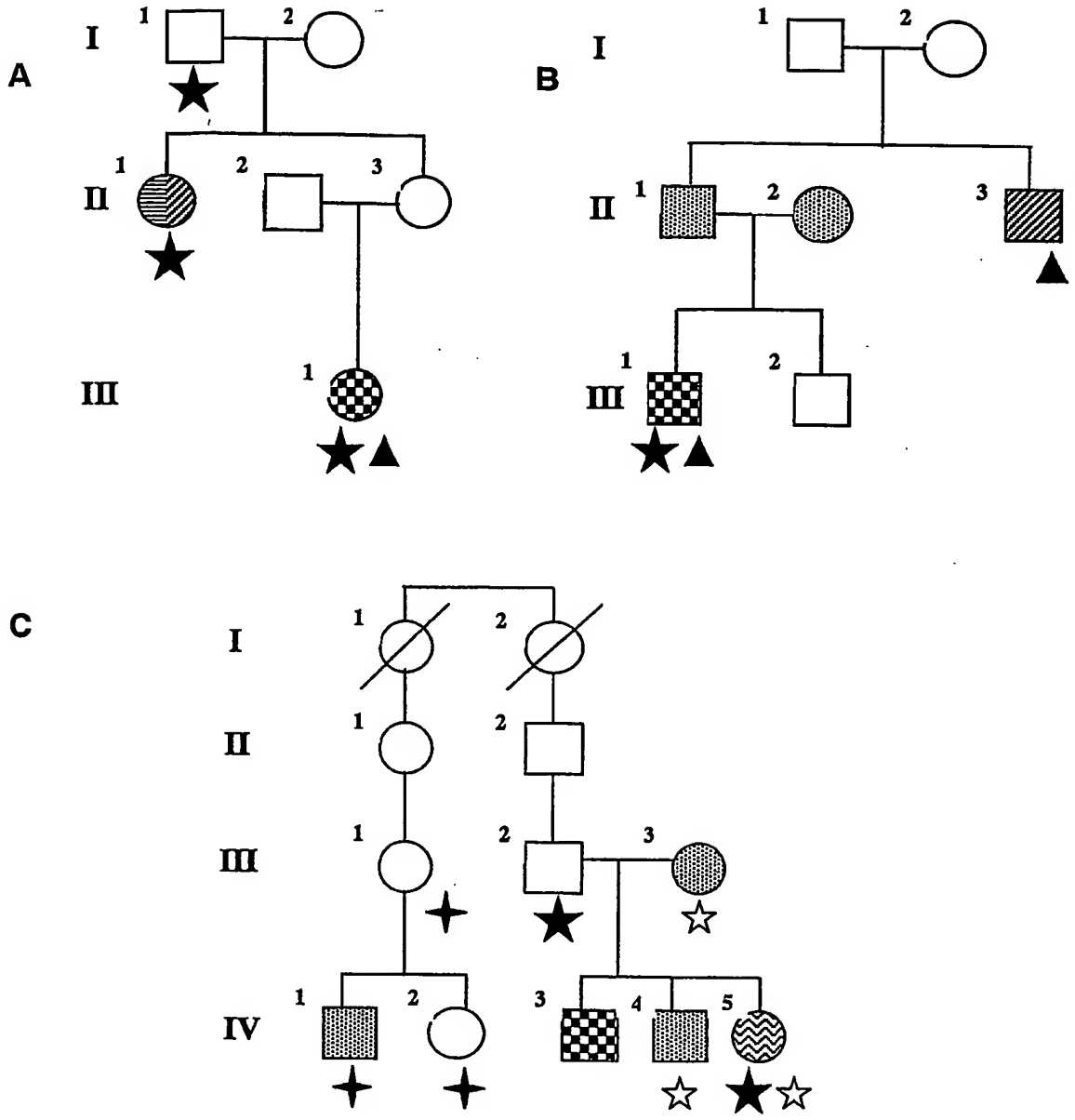
IGE: Inbred Population

D



IGE: Autosomal Dominant

Figure 3


A
I
II
III
B
C
IV
Febrile Seizures
Febrile Seizures Plus
Myoclonic Astatic Epilepsy
Absences
Severe Myoclonic Epilepsy of Infancy
A1067T SCN1A
N43del SCN3A
G1050S SCN8A
Q351X GABRG2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00910

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Neuron 3, pp 327-337 (1989) Shivers et al "Two novel GABAA receptor subunits exist in distinct neuronal subpopulations"; and GenBank Accession No: L08496. Relevant to SEQ ID No 64	1-29; 33-82 and 84-87
X	Mol Pharmacol 49, pp 253-259 (1996) Hadingham et al "Cloning of CDNAs encoding the human gamma-aminobutyric acid type a receptor alpha 6 subunit and characterization of the pharmacology of alpha 6-containing receptors"; and GenBank Accession No S81944. Relevant to SEQ ID No 65	1-29; 33-82 and 84-87
X	J Biol Chem 272, pp 15346-15350 (1997) Hedblom et al "A novel class of GABAA receptor subunit in tissues of the reproductive system"; and GenBank Accession No U95367. Relevant to SEQ ID No 66	1-29; 33-82 and 84-87
X	Nature 385, pp 820-823 (1997) Davies et al "Insensitivity to anaesthetic agents conferred by a class of GABA(A) receptor subunit"; and GenBank Accession No U66661. Relevant to SEQ ID No 67	1-29; 33-82 and 84-87

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00910

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No:II

The international application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept. The fundamental test for unity of invention is specified in Rule 13.2 of the Regulations under the PCT.

"Where a group of inventions is claimed in one and the same international application, the requirement of unity of invention referred in Rule 13.1 shall be fulfilled only where there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical feature" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, make over the prior art."

The problem addressed by the present application is the identification of molecular changes in ion channel subunits to elucidate genetic variants that alone or in combination give rise to an epilepsy phenotype, and to other neuro/physiological disorders associated with ion channel dysfunction. The solution provided by the claims resides in the use of 134 polynucleotide sequences and 39 polypeptide sequences. These sequences fall within the following eleven groups:

1. Sodium channel subunit SCN_A

2. Sodium channel subunit SCN_B

3. Nicotine acetylcholine receptor subunit CHRNA_

4. Nicotine acetylcholine receptor subunit CHRNB_

5. Potassium channel subunit KCNQ

6-11. GABA receptor subunits GABRA; GABRB; GABRD; GABRE; GABRG; GABRP.

The general concept underlying the present application appears to reside in the nucleic acid molecules encoding a mutant or variant ion channel subunit. However, the admitted prior art acknowledges that such nucleic acid molecules encoding the proteins and the proteins per se are known. Further, that the mutations in ion channels have previously been recognised and the activity of these molecules is linked to diseases such as epilepsy, the subject matter of the present invention (see page 46; Example 1 of instant application).

The molecules do share the feature of being examples of mutations and variations in ion channel subunit genes. However, these molecules can only be considered to constitute a special technical feature if these molecules, considered as a single group, make a substantial contribution over the prior art. There is nothing in the instant application to indicate that the isolation of the molecules encoding a mutant or variant in ion channel subunit is inventive. As discussed earlier, the mutations in ion channels have been disclosed and taught in the art, including disclosures in the following selection of documents:

AU-B-56247/96 (and other related documents cited in Example 1, page 46 of present application)
Nature Genetics 19, pp 366-370 (1998) Wallace et al "Febrile seizures and generalized epilepsy associated with a mutation in the Na+ channel β1 subunit gene SCN1B".

Am J Hum Genet 68, pp 859-865 (Mar 2001) Wallace et al "Neuronal sodium channel α1-subunit mutations in generalized epilepsy with febrile seizures plus".